PROGRESS REPORT (IN PART): 1 JULY 2004 TO 30 JUNE 2005

Exploration and field collection for CBT and parasitoids in the Czech Republic, 2004.

Exploration for cherry bark tortrix, *Enarmonia formosana* (CBT) in 2004 was conducted in three major physiographic regions in the Czech Republic. This strategy was based on multiple years (i.e. 2001-2003) of survey and field/lab assessments of the preferred hosts of CBT from central Europe. Sampling was obtained in the areas we earlier explored and determined as CBT positive in southern Bohemia and southeastern Moravia. Our objective was to locate wild and cultivated *Prunus* spp. or banker-trees to obtain CBT infested logs and log-bark samples. This plant material was returned to the Czech Academy of Sciences laboratory for incubation and collection of emerging CBT adults and associated parasitoids. Visual surveys were also made in these regions for CBT host preference, seasonal occurrence and locality data. A total of 30 samples were taken, and less than 10 yielded the required banker-tree value. This term refers to host trees possessing a high number of frass tubes compared with less attacked trees at a given location. The samples were taken at the earliest period of the season soon after fresh frass tubes were detectable in host trees in the target areas. Dr. Stary discovered banker trees in previously unexplored northeastern Moravia. This region is a blend of central European lowland agriculture interfacing with rolling foothills of the eastern Sudeten and western terminus of the Carpathian Mountains. However, the banker trees in this unique regions were detected seasonally too late for CBT rearing in the lab.

Methods

Our approaches for the lab preservation of field collected samples in 2001-3 indicated a restricted seasonal occurrence of parasitoids when related to that for CBT. This phenology may be obscured by some desiccation of the sampled material that may affect CBT emergence and concomitant guild of parasitoids, despite providing adequate relative humidity in the lab. For this reason, a heavily infested CBT tree was experimentally logged. The basal infested area of the log was cut off and placed in an isolation cage with its basal part slightly submerged in water on a plate. The isolation cage was maintained outdoors in 2003 to mimic the field environment as much as possible. The CBT and parasitoid population dynamics of this single trial were judged to be positive and stimulated the 2004 schedule.

Three CBT infested logs were removed from the field and maintained in the described manner in 2004. Some of the targeted trees were found to be highly infested with CBT larvae. Extensive de-barking samples were collected and preserved like the logs.

Host plants

Beside a set of host plants species detected for CBT in the previous years, a new record on apple, *Malus domestica* was determined, besides the prevailing and known CBT hosts of *Prunus avium, P. cerasus, P. serrulata, P. armeniaca, and Sorbus aucuparia.*

Refugia

A remarkable refugium of CBT was determined in an urban forest. In the same site, CBT was detected on *P. avium, P. cerasus, Malus domestica* and *Sorbus aucuparia*, besides the occurrence of CBT on *P. armeniaca, P. persica, P. serrulata* in a broader local environment.

Phenologies

CBT emergence in the field.
The log and log-bark evidence documented an extended CBT emergence period throughout the season. During the end of September there was a CBT adult emergence detection point by the end of October. However, our target site was in the mountains (535 m) of northern Moravia. Elevation and climatic conditions affected the late CBT emergence because no extended emergence was identified in the southern-warmer areas where such an event would corroborate a second generation.

CBT and parasitoids.
A comparison of the emergence dates of CBT and parasitoids from our logs and log-bark samples showed parasitoid emergence began in early spring when CBT larvae begin feeding and actively constructing fresh frass tubes prior to their pupation and adult emergence. Soon CBT and associated parasitoids emerge coincidently, and after mid-July, parasitoid emergence ceases, but CBT displays a protracted emergence until mid-September.

Parasitoid guild

The log and log-bark sampling method again yielded qualitatively and quantitatively numerous parasitoid species and specimens that have all been pinned, labelled and sent to Prof. K. Horstmann, University of Wurzburg, Germany for his authoritative identification. In general terms we observed highest populations of CBT in mixed urban forests, roadside hedges and orchards; whereas, wild cherry species found in nearby mixed forests surprisingly did not yield population levels as in the aforementioned situations.

At the present date, the 2004 parasitoid material is presently being identified and will later be integrated with our earlier reports and publications. Based on our field experience and comparative investigation of our log and log-bark samples from 2003 and 2004, a special paper is under preparation that will supplement the earlier published paper on CBT's parasitoid guild.

Population genetics

Most of our lab emerged adults of CBT for 2004 were preserved in pure ethanol for eventual population genetic studies as was done since 2002.

Summary discussion

The parasitoid complex we've associated with CBT apparently emerges prior to adult CBT emergence and later in the season when another parasitoid emergence coincides with CBT's long flight period. The long life-span of the parasitoid adults seems to contribute to their searching succession for immature hosts, including the CBT. Our research supports the current notion that there are no known parasitoids specific to CBT. Instead, CBT should be considered as one of many prey species attacked by a complex of primarily ichneumonid parasitic Hymenoptera. Possibly, only *Campoplex dubiator* might be more closely specific. The seasonal coincidence of CBT and parasitoids is thus affected by the seasonal peculiarities of parasitods in a broader sense (i.e., strategy). Their over-all season strategies are general and CBT is merely one of their potential host species in the environment.

There are some indications that the parasitoid guild is somewhat site/habitat dependent indicated by sample evidence taken in the same site and date but in subsequent years. Urban environments and cultivated landscapes generally appear to be more favorable for parasitoid sampling; whereas natural stands of forests tend to yield fewer parasitoids. The abundance of CBT preferred host plants and possibly their natural vigor (i.e., pollution effects), may even constrain prey species number and their availability to prey searching parasitoids. CBT may occur on several host plant species in an area and host species switching by CBT populations in a site can be presumed. This may indicate the capability of CBT to alternate between different host plants based on their availability and to display opportunistic behavior that is depend on host species availability (= expansion succession). These various density dependent, behavioral traits for CBT biotypes are outside the scope of this research.

We have concentrated our sampling methods to cover banker trees both in the log and log-bark sampling efforts. Banker trees are also the best sites for parasitoid sampling and rearing. The banker-trees apparently interact with their broader environment. There is an apparent attractiveness that illicit mating interaction of adult CBT in such trees (semiochemicals). Moreover, the wounded sites from de-barking activities and those de-barked sites carefully treated with a sealing spray were observed to manifest an attractiveness because of the presence of fresh frass tubes of early instar larvae in areas quite close to earlier wounds.

We have concluded the following methods are useful for collecting adequate samples of host material hosting a one generation, cambium boring and a non-typical leafroller. General exploration is accomplished in an area that the observer evaluates to center on CBT populations as well as indications for parasitoids. De-barking samples of a smaller size are preferred in this environment. The identified banker trees are now acknowledged to delineate a known area/site. Thus, log and/or log-bark sampling methods should be applied as often as possible within delineated sites.

Biological control of CBT in the Pacific Northwest, 2004.

The rapid expansion of CBT into Oregon slowed in 2004 from movement shown in previous years. One interpretation would be that CBT has reached its latitudinal distribution limit. In Washington state, CBT is known from a western corridor stretching from Canada to Portland, Oregon. Despite its seemingly diminishing southward expansion, there remain breeding populations within its current range. Blaine, Bellingham, Seattle, Olympia and Portland contain strong persistent breeding populations. It is not known whether these populations will remain at low levels or unpredictably expand sometime in the future. Seattle University and the Portland epicenter are two sites representing mid-range and southern leading edge populations of CBT in the Pacific Northwest. Previous years' research indicates a different set of dynamics is occurring in these localities. Monitoring these sites will help to predict future changes of CBT populations in the Pacific Northwest.

Methods

Seattle University.
Seattle University has around 57 ornamental cherry cultivars including 'Yoshino', 'Mt. Fuji' and 'Kwanzan.' Roughly, 60% are infested with CBT. Our project focuses on the six Mt. Fuji cherry trees located along the eastern side of the Quad. These trees are highly infested with CBT. The Quad trees are situated between Bannon Hall and behind a concrete "sitting" wall, approximately three feet high, bordering the Quad. The trees are planted in relatively shallow soil atop underground rooms extending from Bannon Hall. These subterranean spaces undoubtedly moderate winter temperatures. An automated sprinkler system keeps the habitat moist. The area where the trees are planted is also a mix of vegetation including Hydrangeas and groundcovers. The Quad has a central fountain which also raises the humidity level.

Pheromone traps were set out in early summer and checked weekly until September 3. Once establishment of *T. cacoeciae* was determined, three releases of the egg parasitoid were made. Total release numbers range from 19,000 to 190,000 dependent upon the parasitization levels of the surrogate host eggs. A high rate of parasitized CBT eggs were observed during the flight period. Despite the parasitization rate, a large number of new frass tubes were observed in late summer. An additional treatment compatible with *T. cacoeciae* was chosen.

Nematode treatment.
Entomopathogenic nematodes were applied October 13, 2004. The trial tested the efficacy of two species of nematodes: *Steinernema carpocapsae* and *S. feltiae.* One week prior to treatment, infested trees were chosen and brushed so that active larvae could be easily identified. On the day of treatment, forty active frass tubes per tree were selected to test efficacy of the nematodes with and without frass tubes. Twenty of the frass tubes were marked with a dot of White-out® permanent paint and the remaining twenty frass tubes were removed and marked with a dot of neon yellow fabric paint.

The seventeen trees represented six replications for each of the two species of nematodes plus five controls or untreated trees. Nematodes were mixed with a wetting agent and applied with a backpack sprayer at a rate of 1000 nematodes/ml. Spraying time resulted in one million nematodes applied per tree. Following treatment, the trunks were periodically misted with water for approximately two and one-half hours, in order to retain the viability of the nematodes. Treatments were evaluated eight days following the application. Activity was indicated by the presence of frass. Treated versus untreated were analyzed with a *t*-test (Table 2).

Portland epicenter

Less than one mile^2 in total area, the epicenter is located in an affluent neighborhood in the northeast quadrant of Portland, Oregon, two miles from the Willamette River and four miles from the Columbia River. While these rivers provide some additional moisture, they are not equivalent to a coastal setting with its direct maritime influences such as the Seattle site. The epicenter area lies on the edge of the "Irvington Historic District" composed of older established neighborhoods.

Some of the cherry trees in the area were planted over 50 years ago. The trees investigated were primarily street trees or those easily accessed in front yards with the owner's permission. Lawns for the most part are highly manicured and often possess a sprinkler system. Pheromone traps were placed in locations throughout the epicenter to track the flight period of the adult moths. Traps were checked on a weekly basis from May 20 until August 19 when the flight period ended. Numbers of male moths/trap were recorded.

Results

Portland epicenter.

The total number of male CBT moths collected from traps in the Portland epicenter in 2004 showed a 50% decrease in the number collected in 2003 (Table 1). This was predicted in the final report in 2003 (Tanigoshi et al., 2003). This is the first population decline in Oregon since CBT was discovered there in 2000.

Year	OR statewide CBT trap total	Portland epicenter CBT trap number
2000	2	NA
2002	1040	773
2003	1389	915
2004	783	462

Table 1. CBT in Oregon 2002-2004.

Populations of CBT increased 18% from 2002 to 2003 in the epicenter and 78% for the remainder of the state. Conversely from 2003 to 2004, a statewide decrease occurred. CBT populations in the epicenter decreased 56%; elsewhere the statewide decrease was 32%.

A graphic representation of the flight periods from 2002-2004 chronicles the changes in the population. The erratic multiple emergence seen in 2002, have diminished to double peaks in 2003 and 2004 and the time between emergence has increased. The entire flight period appears

to have shifted earlier in the year by approximately 1-2 weeks and ends earlier. As of yet, there is insufficient data to establish this as a trend.

Seattle University
In 2004, a period of 9 weeks trapping at Seattle University garnered 524 moths from an area roughly the size of 1/4 city block, while the Portland epicenter, approximately one mile^2, produced 462 moths during the 13 week flight season.

Trichogramma cacoeciae was found to have established at Seattle University in 2004 after one year of weekly releases during the flight period. In the Portland epicenter *T. cacoeciae* failed to establish after two years of heavy weekly releases during the flight periods from 2002-2004.

Nematode trial.
The nematode trial showed a definite reduction in new tubes (Table 2). These reductions were not as great as those seen in other CBT/nematode trials (Murray et al. 2003). This combination of entomopathogenic nematodes and the parasitoid *T. cacoeciae* may result in an effective, ecologically-friendly method for controlling CBT, particularly at sites which prefer to avoid conventional pesticides such as Seattle University. Data indicates that removal of the frass tubes prior to application increases the effectiveness of the nematodes. *Steinernema carpocapsae* also appears to be slightly more effective than *S. feltiae* but the results are inconclusive and further testing will be required. An evaluation of the infestation should occur in the spring of 2005 to determine the efficacy of this treatment regimen.

Nematode Treatment	% Reduction	
	With frass tubes	Without frass tubes
Steinernema carpocapsae	65%	70%
Steinernema feltiae	61%	66%

Table 2. % reduction in CBT activity

Summary discussion

Oregon data from 2004 indicate the southward expansion has slowed. It is not known whether this is temporary or permanent. The persistent hotspots in Washington suggest that these areas can exist for years. Observations in the epicenter at the end of the flight period were disturbing. New frass tube development appeared to be increasing from those observed earlier in the season. This could indicate the population was rebounding without the presence of *T. cacoeciae* or that the population was capitalizing on the early emergence by producing a partial second generation. Evidence of this was observed at the Seattle site with discovery of late pupal exuviae in September and the discovery of an adult CBT at the time of the nematode trials in mid-October.

Parasitized eggs of CBT were not discovered at Seattle University until 18 June 2004. Parasitized eggs were collected and cultured, resulting in emergence of *T. cacoeciae*. Augmentative releases of *T. cacoeciae* at Seattle University did not take place until July, 2004. *Trichogramma cacoeciae* has therefore established at Seattle University. None of the egg sentinels set out in 2003 resulted in parasitization and 2004 CBT eggs exhibited no parasitization until the June 18 discovery. This suggests the establishment of *Trichogramma* was recent and most likely attributable to the massive augmentative releases in 2003.

Coincidentally, at the same time *T. cacoeciae* emerged, large numbers of *Recurvaria nanella* (Lepidoptera: Gelechiidae) were collected in the CBT pheromone traps. These moths spend the winter as larvae and thus are not the alternative hosts for *T. cacoeciae*, which overwinter in eggs. These moths may be useful as "indicators" however, to predict the appearance of *T. cacoeciae*, whose true alternative host remains unknown. If this late appearance of *T. cacoeciae* is normal, they will not impact CBT's first flight. Typically biological control organisms do not reach effective numbers until after the pest has already surpassed the economic threshold. Therefore, even if *T. cacoeciae* establishes in an area, the CBT first flight may continue to require augmentative releases of *T. cacoeciae*.

Future direction

Depending on available funds, the Portland epicenter should be followed for a fourth year to determine the effects of suspending *T. cacoeciae* releases during 2004. This may also help to determine whether the 2003 drought was the prominent factor in the decrease of CBT in the epicenter or whether it was the result of the two years of parasitoid releases. A spring evaluation of the Seattle University site will help to determine efficacy of the fall nematode application. The evaluation will help develop a treatment strategy for the University's non-chemical pest management program.

Literature cited

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WASHINGTON STATE UNIVERSITY: Assurance Statement(s)

Investigator: Lynell K. Tanigoshi	**Phone: 360-576-6030**
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Project Title: Implementing Biological Control of the Cherry Bark Tortrix	
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Biosafety and Chemical Safety

† includes transgenic plants; * includes livestock pathogens and toxins

☒Project does not involve recombinant DNA/Vectors/Plasmids; Infectious/Select Agents; Carcinogens, Mutagens, or Teratogens; EUP's

Project involves:

☐Recombinant DNA/Vectors/Plasmids†
☐Infectious/Select Agents*
☐Carcinogens, Mutagens, or Teratogens
☐EUP (Experimental Use Pesticides)

MUA #: (if known)

Care and Use of Animals

☒Project does not involve vertebrate animals. ☐Project involves vertebrate animals.

ASAF #: (if known)

Protection of Human Subjects

(includes the use of human tissue or bodily fluids)

☒Project does not involve human subjects. ☐Project does involve human subjects.

IRB #: (if known)

Use of Radioactive Materials or Radiation Equipment

☒Project does not involve the use of radioactive materials or radiation equipment.

☐Project does involve the use of radioactive materials or radiation equipment

Conflict of Interest

Conflict of Interest definitions and questions (www.ogrd.wsu.edu/coi_dq/)

☒Project does not involve a Conflict of Interest

☐Project does involve a Conflict of Interest